Microbial Community and Environmental Factors Affecting Copper Complexation in a Navy Harbor

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LONG-TERM GOAL

Our long term goal is to understand the interactions between microorganisms and trace metals in estuaries that are heavily utilized by Naval operations. We are particularly interested in the production of high-affinity, copper-complexing ligands by microbial populations in response to elevated copper concentrations.

OBJECTIVES

Strong, dissolved, copper-complexing ligands are known to control copper speciation and bioavailability in most marine waters. We are testing the hypothesis that metal-responsive production of such ligands occurs in the Elizabeth River estuary, and that picoplankton and bacterioplankton produce the ligands. Recent studies utilizing cultures of marine picoplankton (*Synechococcus*) and bacterioplankton (*Vibrio*) have demonstrated that, when the cultures are exposed to elevated copper concentrations, these microbes produce copper-complexing ligands having copper-binding strengths similar to those found in marine waters. The primary objective of our study is to extend these observations to field conditions and natural assemblages of estuarine microorganisms in a Navy harbor. An additional objective is isolation and further characterization of the strong, copper-complexing ligands produced by microbes.

APPROACH

During the first year of this project we carried out preliminary incubations of unfiltered water samples to determine the optimum copper concentration (within realistic limits) and the time required to elicit detectable production of copper-complexing ligands by microbial populations at two sites on the Elizabeth River. One site was located adjacent to Pier 12 at the Norfolk Naval Base (Sewell's Point) and the other was located adjacent to the Norfolk Naval Shipyard on the Southern Branch of the Elizabeth River. Navy clearance was required and obtained for placing our incubation apparatus (moored array holding up to 24, two-liter incubation bottles) at the naval base site. Trace metal clean

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sampling procedures were utilized to collect water from each site and ligand concentration and conditional stability constants were measured. The incubation apparatus was designed, constructed and field-tested. In preliminary incubations we measured changes in concentrations, and conditional stability constants of the ligands produced using voltammetric methods; the numbers of bacterioplankton, picoplankton, microzooplankton and phytoplankton, and frequency of dividing cells, at varied copper concentrations and exposure times. On the basis of these preliminary studies, an effective copper concentration and incubation time for stimulation of ligand production were selected. In subsequent experiments we determined the effect of the metabolic poison, sodium azide, and filtration to remove microbial cells and particulate material on ligand production in the presence and absence of elevated copper, and the effect of sodium azide on copper ligand determination.

WORK COMPLETED

Sites were selected and necessary clearances were obtained for our experiments at Navy-controlled stations.

Water samples were collected from the sites and copper ligand concentrations and conditional stability constants were measured.

Preliminary studies were performed to determine the required copper concentration and incubation time to elicit detectable changes in ligand concentration in water samples.

Evaluation of a metabolic inhibitor for use in these studies was completed.

The *in situ* incubation apparatus was designed, constructed and field-tested.

Initial experiments were successfully completed at both sites to determine the effect of an elevated copper concentration and addition of a metabolic inhibitor on ligand production by the intact microbial community.

RESULTS

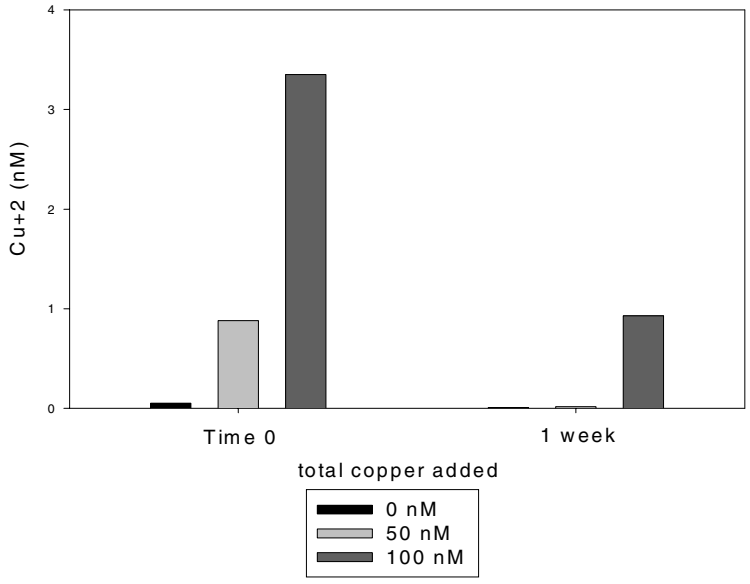
An effective apparatus for in situ incubation was designed and successfully field-tested. The moored array holds 24, two-liter polycarbonate incubation bottles at a constant depth of one meter below the surface. It is buoyed by floats at each corner and moored to the bottom by a line attached to a 15 inch mooring buoy at the top and a 150 lb. weight at the bottom. The array moves freely up or down the mooring line with the tide maintaining the bottles at a constant depth.

Populations of picoplankton, bacterioplankton, phytoplankton and zooplankton were relatively stable or decreased slightly throughout the duration of the experiments, indicating that no major perturbation of community structure was induced by the experimental conditions. Similar trends were observed in most plankton components. Picoplankton at the Norfolk Naval Shipyard site were less affected by copper additions than the picoplankton at the Naval Base site in the July, 1999 experiment.

Evaluation of the compatibility of metabolic inhibitors with the analytical methods used to determine copper speciation and ligand concentrations showed that sodium azide did not interfere with the

measurements. Azide was utilized to inhibit microbial activity in order to determine whether observed increases in ligand concentration resulted from metabolic processes.

In a time series utilizing copper additions of 50 and 100 nM, and sampling times of 0 and 168 hours, copper-complexing ligand concentrations were observed to increase in a dose-dependent fashion when copper was added to water samples containing intact, natural microbial communities. Based on the results of this experiment, an elevated, but environmentally realistic, copper concentration (100 nM) and incubation time (one week) for stimulation of ligand production were selected. Under these conditions the concentration of copper-complexing ligands increased, causing the bioavailable form of copper, the free Cu^{2+} ion, to decrease dramatically (Figure 1).



1. Free Cu^{2+} ion concentrations in unfiltered water samples from the Norfolk Naval Base study site amended with copper and incubated under ambient conditions for one week. Reduction in the free Cu^{2+} ion concentration was caused by increased concentrations of strong, copper- complexing ligands in the samples. Since the Cu^{2+} ion is the bioavailable form of copper, these data indicate substantial detoxification has occurred during the incubation period.

An experiment was performed at both stations (Naval Base and Norfolk Naval Shipyard) in July 1999 to determine the effect of sodium azide (15 mM) and filtration (0.22μM) on ligand production in the presence and absence of 100 nM copper additions. Ligand concentration measurements and data analyses on the samples generated from this experiment are still in progress.

IMPACT/APPLICATION

This work will clarify the influence of microbial activity on copper speciation and bioavailability, and determine whether microbial populations actively produce copper-complexing ligands in response to

increased copper concentrations *in situ*. If this hypothesis is validated it will impact our thinking about the fate and effects of copper in estuarine ecosystems and naval harbors. A better understanding of how estuarine systems can respond to copper influx will allow for a more rational basis for setting concentration limits on effluents while protecting the health of the estuary.

RELATED PROJECTS

Concurrent, ONR-funded projects entitled "Interaction among chemical speciation, algal accumulation, and sediment-water cycling of toxic metals in a major US Naval harbor (Elizabeth River, VA)", P.I.s J.R. Donat, and D. Burdige (Old Dominion University), and W.Sunda and S. Huntsman (NMFS/NOAA), are examining factors controlling algal metal uptake and accumulation and the importance of sediments as a source of metals and metal chelators to the overlying water. Since these projects are closely related, are examining the same site, and utilize some of the same methodologies as our project, all PIs are coordinating their efforts and sampling times to maximize the amount of information obtained. During July 1999 we coordinated a sampling cruise on the Elizabeth River with deployment of our *in situ* incubation arrays at the two sites. The selected sites for our incubation arrays were within the cruise track. Data from these concurrent studies will be integrated to provide additional insight into the processes controlling microorganism trace-metal interactions in the Elizabeth River.

The analytical techniques for determination of copper ligand concentration and speciation utilized in this project are also being used (supervised by Donat) in a related project in Gordon's laboratory that is investigating copper resistance mechanisms in marine bacteria (*Vibrio spp*). Specifically we are examining copper accumulation in wild-type and copper-sensitive transposon mutants of *Vibrio parahaemolyticus.*

PUBLICATIONS

Bruland, K.W., E.L. Rue, P. Kozelka, **J.R. Donat**, J.W. Moffett, and S.A. Skrabal. 1999. An intercomparison of analytical approaches to determine the chemical speciation of dissolved copper in an estuarine sample from Narragansett Bay, Rhode Island. Analytica Chimica Acta**,** in press.

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